INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Development of Fluorescent Nucleobase Analogues
- Intrinsically labelled nucleic acids for molecular binding investigations

Mattias Bood
Institutionen för kemi och molekylärbiologi
Naturvetenskapliga fakulteten

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Abstract

This thesis focuses on the design, synthesis and utilization of fluorescent nucleobase analogues (FBAs). FBAs are an important class of compounds, used in the research of nucleic acids. The class of canonical FBAs, *i.e.* like the natural nucleobases, are of special interest as they can replace the natural nucleobases without significantly perturbing the overall structure and biological function of the nucleic acid. The overarching goal of the project was to establish a molecular binding interaction assay based on novel FBAs, to study ligand binding to oligonucleotides.

This thesis starts with explaining the design rationale behind the class of quadra- and penta-cyclic adenine analogues, followed by the developed synthetic methods to such constructs. The developed synthetic scheme was used to prepare a library of over 50 novel multicyclic adenine analogues.

One of the brightest molecules, pA, was incorporated and characterized inside DNA and was found to not perturb the overall structure of duplex DNA significantly. Moreover, pA was characterized as one of the brightest adenine analogues in DNA and RNA at the time of publishing. Follow-up studies revealed that pA can be detected *via* two-photon spectroscopy at a ratio of signal to background as low as five to one, meaning that our developed FBAs are approaching super resolution imaging applications.

Another remarkable compound that was identified from the early screening study was 2CNqA, which just recently turned out to be the brightest FBA in DNA and RNA to date. The interbase FRET (Förster resonance energy transfer) properties were studied of 2CNqA in both DNA and RNA, and the probe accurately reports FRET of at least 1.5 turns of DNA, making it suitable to study changes over short DNA and RNA.

The thesis is concluded with the synthesis, incorporation and characterization of the FRET pair tC^{O}-tC_{nitro} in RNA where they were used to monitor changes from A- to Z-form RNA. Furthermore, the FRET pair was then used to study the antibiotic class of aminoglycosides binding to RNA, faithfully reporting on their relative binding affinity of a pre-microRNA construct.

Keywords: Fluorescent nucleobase analogue, FRET, surface plasmon resonance, isothermal titration calorimetry, DNA, RNA, pre-microRNA, aminoglycoside, binding interaction.

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